Association study of phosphodiesterase genes in the Sequenced Treatment Alternatives to Relieve Depression sample

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A recent study has reported a significant association of variants in phosphodiesterase (PDE) genes with antidepressant treatment outcome in a Mexican American sample. We set out to investigate these findings in a large sample of patients from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study. STAR*D is a longitudinal study of antidepressant outcome in depressed outpatients. We genotyped three single nucleotide polymorphisms (SNPs) in PDE11A (rs1880916), PDE1A (rs1549870), and PDE9A (rs729861) for replication and we also report three additional SNPs in PDE11A (rs3770016, rs4893975, rs6433687) that had been genotyped for a previous study. Single marker analysis of remission within the Hispanic subsamples ($n=268$) revealed no significant evidence of association with markers in PDE11A, PDE9A, or PDE1A. Additional analyses of remission within the total STAR*D sample ($n=1914$) were also largely negative, as were analyses utilizing a narrower definition of remission. Haplotype analyses were carried out with the four PDE11A SNPs we genotyped; these also failed to show significant evidence of association in the STAR*D sample. In conclusion, we could not reproduce the reported association between PDE genes and antidepressant outcome in a sample of participants comparable to that reported previously. We conclude that PDE11A, PDE9A, and PDE1A are unlikely to play an important role in antidepressant outcome in this sample. *Pharmacogenetics and Genomics* **19:235–238** © **2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Major depressive disorder (MDD) is a leading cause of disease burden worldwide [1]. MDD is a common disease with underlying genetic and environmental components that have not yet been clearly elucidated. Although many patients benefit from medications, full remission is achieved only in a minority [2]. In addition, patients respond differently to various treatments [3], some of these variations are attributed to genetic differences [4–7]. Genes associated with treatment outcome may help expose the pathophysiology of MDD and lead to better treatments.

A recent study has reported a significant association of variants in phosphodiesterase (PDE) genes with MDD and treatment outcome in a Mexican–American sample. Evidence for association with antidepressant treatment response was detected with single nucleotide polymorphisms (SNPs) within PDE9A (rs729861) and PDE11A (rs3770018). Remission on antidepressants (fluoxetine or desipramine) was shown to be significantly associated with variations within PDE1A (rs1549870) and PDE11A (rs1880916), with odds ratios of attaining remitter status of 4.6 and 3.2, respectively [8].

Eleven different PDE gene families have already been identified and characterized [9–16]. PDE enzymes hydrolyze intracellular cyclic AMP and/or cyclic GMP, and play an important role in various biological and pharmacological processes [17]. PDE genes are thus reasonable candidates for mediating response to antidepressants and other drugs.

Methods

We set out to investigate these findings in a large sample consisting of 1914 MDD patients from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study [18]. The rationale, methods, and design of the

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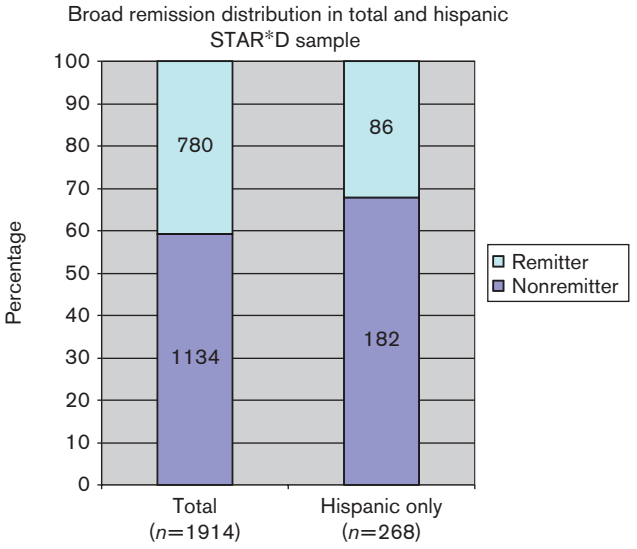
STAR*D study have been detailed elsewhere [19]. In brief, investigators at 14 regional centers across the United States implemented a standard study protocol at 41 clinical sites. Participants provided both written consent and blood samples for the study. Outpatients aged 18–75 years with a baseline Hamilton Depression Rating Scale score of ≥ 14 who met DSM-IV criteria for nonpsychotic MDD were eligible. At the first step of treatment, the selective serotonin inhibitor, citalopram, was offered to all participants. The 16-item Quick Inventory of Depressive Symptomatology-Clinician-rated (QIDS-C16) was obtained at baseline and at each treatment visit, to measure symptom change over time.

Sampling methods, DNA collection, and phenotypic definition and assignment have been described elsewhere [6]. All phenotype definitions and assignments were settled in advance and were assigned before genotyping. We used the broad definition of remission as defined in our previous studies of STAR*D (Fig. 1). Broad definition included probable remitters (QIDS-C16 scores = 6 or 7 at their last treatment visit) and nonremitters included probable nonremitters (QIDS-C16 scores = 8 or 9). The narrow definition excluded participants with QIDS-C16 scores between 6 and 9. Our broad definition corresponds closely to that used in the study by Wong *et al.* [8], given that they defined remission as having a final 21-item Hamilton Depression Rating Scale score of less than 8.

Fig. 1



Percentage of treatment outcome phenotypes of all participants and Hispanic subset. Participants who completed at least 6 weeks of treatment with citalopram were assigned a remission phenotype based on the Quick Inventory of Depressive Symptomatology-Clinician-rated score at the last treatment visit. Broad phenotypic definition of treatment outcome grouped probable remitters under remitters and probable nonremitters with nonremitters. STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

We analyzed our data in the total STAR*D sample ($n = 1914$) and as the Wong *et al.* [8] report was based on an Hispanic sample, we also analyzed our data in the subgroup of self-reported Hispanics ($n = 268$). The power to detect at P value of less than 0.05, an effect as large as that reported in Wong *et al.* [8] (odds ratio = 3.2), is 100% in the total sample and 98% in the Hispanic subset. If the actual effect size is as low as the lower reported bound of the 95% confidence interval (odds ratio = 1.27), then we have 86% power to detect the effect in the total sample and 15% power to detect the effect in the Hispanic subset. Power was calculated under a dominant model using Genetic Power Calculator [20].

We selected rs1549870 from PDE1A (best marker associated with fluoxetine remission), rs1880916 from PDE11A (best marker in PDE11A associated with fluoxetine and fluoxetine or desipramine remission) and rs729861 from PDE9A (best PDE9A marker associated with MDD) for replication. Wong *et al.* [8] reported evidence for association with treatment outcome at rs1549870 ($P \leq 0.005$) and rs1880916 ($P \leq 0.04$) and evidence for association with depression at rs729861 ($P = 0.0006$). Three additional SNPs in PDE11A (rs3770016, rs4893975, rs6433687) that were genotyped in an earlier study were also added to the analysis. Of the four PDE11A SNPs selected, three are located in the first haplotype block. Genotyping these three SNPs (rs3770016, rs4893975, rs6433687) allows detection of haplotype associations within the least common haplotype block, but cannot distinguish between the two most common blocks.

Genotyping was done using Taqman allele discrimination assay. SNP probes were ordered form Applied Biosystems (ABI, California, USA) and assays were performed using a modification of the manufacturer's suggested procedures. Likelihood ratio χ^2 tests with 1-degree of freedom (Cocaphase v2.404) and 2-degrees of freedom (Unphased 3.0.4) were used to analyze frequencies of alleles and genotypes, respectively. PDE11A haplotypes were analyzed using a likelihood ratio χ^2 tests with 1-degree of freedom (Unphased 3.0.4). Hardy–Weinberg equilibrium and marker–marker linkage disequilibrium were calculated by using HaploView 4.0 [21].

Results

The results were largely negative (Table 1). No SNPs were significant in the total sample or in the Hispanic subset, either in the genotypic test or the allelic test analyses. Additional analyses of remission within the total STAR*D sample ($n = 1914$) were also largely negative, as were analyses using the narrow definition of remission. Haplotype analyses were also carried out with the four PDE11A SNPs we genotyped. These analyses also failed

Table 1 Genotypic results SNPs in PDE11A, PDE9A, and PDE1A in both total STAR*D and Hispanic subset samples

		Total STAR*D ($n=1914$)			Hispanics ($n=268$)		
SNP	Genotypes	Remitters (%)	Nonremitters (%)	*P* value	Remitters (%)	Nonremitters (%)	*P* value
rs4893975	AA	54 (6.0)	41 (8.2)	0.321	5 (4.8)	5 (6.3)	0.809
	GA	267 (29.8)	146 (29.1)		26 (24.8)	17 (21.5)	
	GG	575 (64.2)	315 (62.8)		74 (70.5)	57 (72.2)	
rs6433687	AA	32 (3.5)	19 (3.7)	0.844	3 (2.8)	1 (1.3)	0.755
	GA	232 (25.6)	137 (26.9)		24 (22.4)	18 (22.5)	
	GG	641 (70.8)	353 (69.4)		80 (74.8)	61 (76.3)	
rs3770016	AA	64 (7.1)	33 (6.5)	0.794	4 (3.7)	4 (5.1)	0.834
	GA	273 (30.2)	161 (31.8)		25 (23.2)	20 (25.3)	
	GG	567 (62.7)	313 (61.7)		79 (73.2)	55 (69.6)	
rs1880916	AA	41 (4.5)	31 (6.1)	0.421	3 (2.8)	3 (3.8)	0.775
	GA	318 (34.8)	176 (34.6)		31 (28.7)	26 (32.5)	
	GG	555 (60.7)	302 (59.3)		74 (68.5)	51 (63.8)	
rs1549870	AA	10 (1.1)	2 (0.4)	0.238	0 (0.0)	1 (1.3)	0.356
	GA	149 (16.6)	93 (18.5)		14 (13.1)	8 (10.1)	
	GG	738 (82.3)	409 (81.2)		93 (86.9)	70 (88.6)	
rs729861	GG	234 (25.9)	147 (28.9)	0.405	42 (38.9)	33 (41.3)	0.095
	GA	447 (49.6)	248 (48.8)		54 (50.0)	30 (37.5)	
	AA	221 (24.5)	113 (22.2)		12 (11.1)	17 (21.3)	

PDE, phosphodiesterase; SNPs, single nucleotide polymorphism; STAR*D, Sequenced Treatment Alternatives for Depression study.

to show any significant evidence of association in either the total sample or the Hispanic subset.

Two of the SNP genotype distributions were in Hardy–Weinberg disequilibrium in the whole sample (rs4893975 and rs3770016). These SNPs were genotyped further in 384 healthy controls, to confirm that Hardy–Weinberg equilibrium deviation was not a technical issue, and to explore the possibility that these deviations might be disease related as suggested by Wittke-Thompson *et al.* [22]. If only non-Hispanic whites are analyzed for rs3770016, the Hardy–Weinberg deviation disappears but the deviation remains for rs4893975. We further analyzed this marker using major depression as the phenotype (vs. healthy controls) and found that the Hardy–Weinberg equilibrium deviation was driven by cases. Therefore, we conclude that although the Hardy–Weinberg equilibrium deviations in rs3770016 are a Hispanic and White admixture problem, the deviation in rs4893975 suggests that variation in PDE11A may be associated with major depression.

Conclusion

We could not reproduce the reported association between the PDE SNPs and antidepressant outcome in a sample of the patients comparable to that reported earlier; furthermore, there was no evidence of association even with a much larger sample. However, although our data do not support a PDE11A role in antidepressant outcome, further study is warranted to determine whether PDE11A is associated with major depression. The STAR*D study was based on citalopram treatment, whereas the study reported by Wong *et al.* [8] used fluoxetine and desipramine. Although citalopram and fluoxetine both bind to the serotonin transporter, other differences in the drugs themselves may explain the difference in results.

This study has several limitations: (i) although STAR*D was not designed for pharmacogenetic studies, it provides the largest cohort of patients treated with a single drug who were prospectively followed and provided DNA and consent for genetic studies. (ii) Our group (and others) have conducted a number of different pharmacogenetic analyses on this sample [5–7]. (iii) Medication adherence in STAR*D was limited to patient's report, no measurements were made regarding plasma drug levels during the time of treatment. (iv) Concomitant antidepressant drugs were prescribed by the STAR*D protocol, and although trazodone (up to 200 mg) was used as a hypnotic, the dosage allowed does not provide a significant antidepressant effect. (v) Participants of STAR*D were not screened for axis II disorders.

We conclude that the SNPs reported as having associations in PDE11A, PDE9A, and PDE1A are unlikely to play an important role in antidepressant outcome in this sample. A recent report by Teranishi *et al.* [23], which also used the STAR*D sample, reached a similar conclusion.

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